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Done

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	09/234,532	
Group Art Unit:	1623	**RESPONSE TO OFFICE ACTION**
Filing Date:	1/21/99	
Examiner:	Howard Owens, Jr.	
Inventor:	Dr. Alfred T. Sapse	

Title: COMPOSITION OF ANTI-HIV DRUGS AND ANTI-CORTISOL COMPOUNDS AND METHOD FOR DECREASING THE SIDE EFFECTS OF ANTI-HIV DRUGS IN A HUMAN

Box AF
Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8 (A)

Date of Deposit: September 19, 2000

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as first-class mail in an envelope addressed to Box AF, Assistant Commissioner for Patents, Washington, D.C. 20231.

Jody K. Prince

Dear Sir:

The following remarks and Declaration under 37 C.F.R. §1.132 are in response to the Examiner's Final Office Action mailed on 7/21/00. Applicant hereby requests reexamination and reconsideration of the application, in view of the following remarks and Declaration under 37 C.F.R.§1.132.

#15

IN THE UNITED STATES DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

In re Application of:	Sapse	Attorney Docket No.	1398-002
Serial No.:	09/234,532	Group Art Unit:	1623
Filing Date:	January 21, 1999	Examiner:	Howard Owens, Jr.

Title: COMPOSITION OF ANTI-HIV DRUGS AND ANTI-CORTISOL COMPOUNDS AND METHOD FOR DECREASING THE SIDE EFFECTS OF ANTI-HIV DRUGS IN A HUMAN

Box Non-Fee Amendment
Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. VASSILIOS PAPADOPOULOS UNDER 37 C.F.R. § 1.132 IN SUPPORT OF PATENTABILITY

I, Dr. Vassilios Papadopoulos, being duly cautioned, declare as follows:

1. I am a professor of cell biology, pharmacology and neuroscience in the department of Pharmacology & Neurosciences at Georgetown University Medical Center.

2. I have been a professor at Georgetown University Medical Center since 1988.

3. I have overseen and been in direct control of recent experimentation involving the inhibition of cortisol production in human adrenal tumor cells.

4. The results of a study conducted at Georgetown University Medical Center and released in May 2000, show that a combination of procaine, zinc sulfate heptahydrate and ascorbic acid has a cortisol inhibition effect that is more elevated than the cortisol inhibition effect of each ingredient administered separately (a synergistic effect). The combination of procaine, zinc sulfate heptahydrate and ascorbic acid lead to a clear and extremely significant cortisol inhibition.

5. The results obtained from the above-mentioned study were unexpected results.

6. A copy of the test methodology employed in the above-mentioned study is attached as Exhibit A.

7. All statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

8. I have been warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

9/19/00

Date

Dr. Vassilios Papadopoulos

Methods:

Cell culture - The H-295R human adrenal carcinoma cells used were a gift from Dr. W.E. Rainey (University of Texas Southwestern Medical Center, Dallas, TX). The cells were maintained in Dulbecco's Modified Eagle's/Ham's F-12 media supplemented with ITS plus [insulin (1 μg/ml), transferrin (1 μg/ml), selenium (1 ng/ml), linoleic acid (1 μg/ml), and BSA (1.25 mg/ml)], and 2.5% Nuserum (type I) as described (1,2). For experiments H-295R cells cultured in 48 well culture dishes (Costar, Corning, NY) were treated for 48 hours with the indicated amounts of the compounds. At 48 hours, 1 mM of the cAMP hydrosoluble analogue dibutyrylcAMP (dbcAMP) was added for another 48 hours. In separate experiments, after the initial 48 hour treatment the cells were washed and then incubated with fresh materials including 1 mM dbcAMP. No significant differences between these two experimental protocols could be seen. At the end of the treatment the media were saved for cortisol measurement. Cells were treated with 0.1 M sodium hydroxide for protein determination.

Assays - Cortisol levels, as an index of steroid production by these cells (1,2), were measured in the culture media by radioimmunoassay using conditions provided by the supplier of the antisera (ICN, Costa Mesa, CA). Microgram amounts of protein were quantified by the dye-binding assay of Bradford (3) with gamma-globulin as standard.

Statistics - Four independent experiments were performed. Each experiment was performed in triplicates. Results were expressed as the means $\pm$ S.D. Statistical analysis was performed by ANOVA followed by the *Student-Newman-Keuls* test or the *Dunnett* multiple comparisons test using the Instat (v.2.04) package from GraphPad, Inc. (San Diego, CA).

References:

1. Rainey WE, Bird IM, Sawetawan C, Hanley NA, McCarthy JL, McGee EA, Webster R. Mason JI 1993 Regulation of human adrenal carcinoma cell (NCI-H295) production of C19 steroids. *J Clin Endocr Metab* 77:731-737
2. Amri H, Ogwuegbu SO, Boujrad N, Drieu K, Papadopoulos V 1996 *In vivo* regulation of the peripheral-type benzodiazepine receptor and glucocorticoid synthesis by the *Ginkgo biloba* extract EGb 761 and isolated ginkgolides. *Endocrinology* 137, 5707-5718
3. Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal Biochem* 72:248-254